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13. Abstract

The *clusterin* gene encodes a cytoprotective chaperone protein that promotes cell survival. *Clusterin* is expressed in a variety of cancers including prostate, increases in response to apoptotic stimuli, and in pre-clinical models confers a resistant phenotype. OGX-011 is a 2nd generation antisense complimentary to *clusterin* mRNA that inhibits expression of *clusterin* in xenograft models and thereby increases sensitivity to therapy. To evaluate OGX-011 as a potential treatment in humans, we have undertaken this Phase I/II study to evaluate the clinical, pathologic and biologic effects of OGX-011, in combination with neoadjuvant hormone therapy (NHT) in patients with prostate cancer and high risk features prior to radical prostatectomy. The primary objective of the phase I study was to determine phase II dose based on target regulation effect in addition to standard toxicity parameters. The phase II component of this trial will assess the effects of combined NHT and OGX-011 on pathologic complete response. **Progress:** 25 patients were enrolled to 6 cohorts with doses of OGX-011 up to 640mg delivered. Toxicity was limited to grade 1/2, including fevers, rigors, fatigue and transient AST and ALT elevations. There were no dose-limiting toxicities. Plasma PK analysis showed linear increases in AUC and Cmax with a t1/2 of approximately 2h. Prostate tissue concentrations of OGX-011 increased with dose, and tissue concentrations associated with preclinical effect could be achieved. Dose dependent decreases in prostate cancer cell *clusterin* expression were observed by QRT-PCR and immunohistochemistry (IHC). At 640mg dosing, *clusterin* mRNA was decreased to a mean of 8% (SD=4%) compared with lower dose levels and historical controls as assessed by QRT-PCR on laser captured microdissected cancer cells. By IHC, mean % cancer cells staining 0 intensity for *clusterin* protein at 640mg dosing was 54% (SD=24%) compared with 2-15% for lower dose levels and historical controls. Conclusions: OGX-011 is well tolerated and can inhibit *clusterin* expression in primary prostate cancers. The recommended phase II dose for OGX-011 is 640mg based on pharmacokinetic parameters and target regulation results. The Phase II portion of this study is in preparation with planned accrual to start in October 2004.

14. SUBJECT TERMS
Prostate cancer, clusterin, antisense oligonucleotide, methoxyethyl gapmer

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INTRODUCTION

The *clusterin* gene on chromosome 8 encodes a chaperone protein which has been implicated in a variety of physiologic processes. Also known as *Testosterone repressed prostate message-2* [TRPM-2], or *sulfated glycoprotein-2*, *clusterin* is associated with numerous tumors including prostate [1], neuroblastoma [2], breast [3], lymphoma [4], urothelial [5] and renal cell carcinoma [6], and with various pathologic conditions including Alzheimer's [7] and nephrotoxic injury [8]. Clusterin levels increase dramatically during castration-induced apoptosis in rat prostate epithelial cells [9], in androgen dependent Shionogi tumors [10], and human prostate cancer CRW22 [11] and PC82 [12] xenografts. In human prostate cancer, clusterin levels are low or absent in most untreated hormone-naive tissues, but increase significantly within weeks after neoadjuvant hormone therapy [13]. Because clusterin binds to a wide variety of biological ligands [14,15], and is regulated by transcription factor HSF1 (heat shock factor 1) [16], the emerging view suggests that clusterin functions similarly to heat shock protein to chaperone and stabilize conformations of proteins at time of cell stress. Indeed, clusterin is substantially more potent than other HSP's at inhibiting stress-induced protein precipitation [17]. Significant differences exist, however, in amino acid sequence analysis which suggests that clusterin is a unique protein without any closely related family members yet identified.

Experimental and clinical studies in prostate cancer implicate clusterin with AI progression and with playing a protective role against apoptotic cell death from androgen withdrawal, chemotherapy and radiation [10,18.19,20]. OGX-011 is an ASO complementary to the *clusterin* mRNA. OGX-011 incorporates a phosphorothioate backbone with second-generation chemistry in the form of 2'-O-Methoxytheyl modifications to the 4 bases on either end of the 21-mer molecule. Such "gap-mer" modifications maintain the improved tissue pharmacokinetic profile of the second-generation chemistry but preserves high affinity for target mRNA and recruitment of RNase H necessary for activity. In pre-clinical models, OGX-011 improves the efficacy of chemotherapy, radiation, and androgen withdrawal by inhibiting expression of clusterin and enhancing the apoptotic response [10,19,20,21]. Furthermore, because of the second-generation chemistry and enhanced tissue half-life of OGX-011, more relaxed dosing schedules are possible while maintaining biologic efficacy of target inhibition. Rather than the prolonged continuous infusions of first generation phosphorothioate molecules that are usually employed, pre-clinical studies suggest that only weekly infusional dosing or less is required to maintain tissue levels of OGX-011 and target inhibition of clusterin [21], which is much more acceptable for patients in terms of tolerance and repeated administration.

To evaluate OGX-011 as a potential treatment in humans, we have undertaken this Phase I/II study to evaluate the clinical, pathologic and biologic effects of OGX-011, in combination with neoadjuvant hormone therapy in patients with prostate cancer and high risk features prior to radical prostatectomy. This primary objective of the phase I component of this trial is to define a recommended phase II dose of OGX-011 based on toxicity and maximal biologic effect. Secondary aims are to determine toxicity, the serum and tissue pharmacokinetic profile and measure evidence of OGX-011 effect on clusterin expression in tumor and peripheral blood mononuclear cells, and clusterin serum levels. The primary objective of the phase II component of this trial will assess the effects of combined neoadjuvant hormone therapy and OGX-011 for 3 months prior to radical prostatectomy on pathologic complete response.

A significant difficulty in the development of targeted therapy agents like OGX-011 is the determination of a biologically effective dose. The biologically effective dose can often be significantly different from that of the maximally tolerated dose, the usual endpoint in classically designed phase I trials. This study's phase I design allows for a determination of an optimal biologically effective dose based on the target of interest (i.e. clusterin) within target tissue itself (i.e. prostate cancer) allowing for confidence in moving forward in phase II trials of the agent.

BODY

TASK 1. STUDY INFRASTRUCTURE PREPARATION

- *Health Canada (Therapeutic Products Program) Investigational New Drug Submission*
- *Case Report Forms*
- *Medical and data monitoring*
- *Institutional Review Board*

All preparatory steps have been completed. Federal regulatory approval was given on 4 October 2002 (File Number 9427-N0711-98C). Initial University of British Columbia Research Ethics Board approval was granted October 24, 2002 (Number C02-0430), and HSRRB approval granted December 2002 (Number A-11279). Medical and Data monitoring and Case Report Form creation services were contracted with the National Cancer Institute of Canada - Clinical Trials Group.

TASK 2. PHASE I TRIAL

- *Patient enrollment*
- *Protocol treatment and dose escalation with OGX-011*
- *Define recommend phase II dose based on toxicity, serum and tissue pharmacokinetic and pharmacodynamic data*

TASK 4. SUPPORTING AND TRANSLATIONAL STUDIES

- *Serum pharmacokinetics*
- *Tissue pharmacokinetics*
- *Clusterin expression – prostate/tumor, mononuclear cells, serum*

- *Comparative molecular marker analysis in pathologic specimens*

Patients having localized prostate cancer with high-risk features and candidates for prostatectomy were enrolled to this dose escalation trial. OGX-011 was given by IV infusion over 2 hrs at a starting dose of 40mg on days 1, 3, 5, 8, 15, 22, and 29. Buserelin and flutamide were started on day 1. Prostatectomy was performed day 30-36. OGX-011 plasma PK and prostate tissue concentrations were determined. Prostate tissues, lymph nodes, and serial samples of peripheral blood mononuclear cells and serum were assessed for *clusterin* expression. The first patient was accrued December 2002, as soon as regulatory and ethical approval had been obtained. As of July 15 2004, 25 patients were enrolled to 6 dose levels on the phase I component of this trial and completed their 3 months of follow-up post radical prostatectomy. There have been no dose limiting toxicities. All patients have had their prostatectomy's performed, with all patients completing protocol therapy and protocol defined follow-up. Patient characteristics are listed in Table 1.

Treatment was well tolerated with no unexpected toxicities, no serious adverse events and no dose limiting toxicities. The most usual toxicity were that of a flu-like syndrome occurring after the infusion of the OGX-011 with patients experiencing fevers, chills and myalgias, which was transient and resolved spontaneously. These usually occurred during the day 1 and 3 infusions only and did not re-occur. Other side effects including transient elevations in hepatic transaminases, again during the first week of treatment, and then resolving despite continued therapy with the OGX-011. Tables 2 and 3 list the Hematologic and non-Hematologic adverse events (graded according to NCI CTCAE v2.0 criteria) that were considered possibly, probably or definitely associated with OGX-011 therapy.

Plasma concentrations of OGX-011 were measured at various time points for pharmacokinetic profiling on Day 1 and repeated on Day 29 to evaluate possible repetitive dosing effects. Plasma pharmacokinetic profile of OGX-011 was as predicted from animal studies and clinical studies with other antisense oligonucleotides with no evidence of significant effect from the multiple dosing schedule (Table 4). Initial half life was approximately 2 hours, and peak concentration (C_{MAX}) (Figure 1, Panel A) and area under the curve (AUC) (Figure 1. Panel B) were linear proportional to dose. More importantly, and more interestingly, concentrations of full length OGX-011 associated with biologic effect *in vitro*, could be achieved in humans prostate tissue (Figure 2). Tissue concentrations increased proportionally to dose.

Clusterin mRNA expression was measured from laser captured microdissected prostate cancer cells from the subjects prostatectomy specimens using quantitative real time PCR (Figure 3). Laser captured microdissected prostate cancer cells were taken from subjects prostatectomy specimens that had not been treated with any neoadjuvant therapy, or from subjects treated with less than 2 months of neoadjuvant hormone therapy served as historical controls. The first two columns in figure 3 represent the historical controls with the first column representing clusterin mRNA expression in prostate cancer cells from subjects without prior neoadjuvant hormone therapy, and the second column representing clusterin mRNA expression in prostate cancer cells from subjects treated with less than 2 months of neoadjuvant hormone therapy, nicely showing the increase in clusterin that occurs in surviving cells after neoadjuvant hormone therapy. In the subjects treated with OGX-011 there was an apparent dose-response relationship with the expression of Clusterin mRNA decreasing with higher dosing of OGX-011. The prostate cancer cells from subjects treated at the 640 mg dosing had 8% of the expression of Clusterin mRNA as compared to historical untreated controls controls.

Protein expression of Clusterin was evaluated using immunohistochemistry. Staining and scoring of prostate cancer cells from representative sections from the prostatectomy specimens were assessed by two pathologists blinded to treatment assignment. Overall score was a composite of staining intensity as well as percentage of prostate cancer cells with that staining. For example, if 50% of cells had an intensity score of 3 and 50% of cells had an intensity score of 0, the overall score was 1.5. Results for overall score are shown in figure 4. An apparent dose response effect is seen with decreased expression of clusterin in prostate cancer cells from patients treated with higher doses of OGX-011. More revealing is the percentage of prostate cancer cells that had 0 intensity scoring (i.e. presumably no clusterin protein expression) as shown in figure 5. Again, a dose-response effect is observed, with an increasing percentage of cells with suppressed clusterin protein expression and somewhat of a plateau in biological effect between the 480 mg and 640 mg dose levels. In addition, the percentage of cells that had suppressed clusterin expression (visual score 0) also increased from baseline (as assessed from the core biopsy specimens) to after treatment with OGX-011 in a dose dependent fashion (figure 6). It must be taken into consideration however, that the magnitude of the suppressive effect of OGX-011 on clusterin protein expression may be under-appreciated given the upregulation of clusterin that normally occurs with neoadjuvant hormone therapy which is not taken into account with this analysis (and hence the use of historical control populations in the previous analyses).

Normal lymph nodes have high expression of clusterin, especially in the germinal center. Similar to the tumor tissue, in normal lymph node tissues taken at the time of radical prostatectomy, there was also an OGX-011 dose dependent inhibition of clusterin mRNA expression (Figure 7) as assessed by QRT-PCR and of protein expression as assessed by immunohistochemistry.

The apoptotic index was also determined from the prostatectomy specimens post treatment. Apoptotic cells were identified using an antibody against ssDNA. As shown in figure 8, there was a trend to an increasing number of apoptotic cells in the prostatectomy specimens with increasing dose of OGX-011.

Expression of clusterin using QRT-PCR was performed in peripheral blood mononuclear cells however the results are uninterpretible due to widely fluctuating results at baseline (three baseline samples taken at different time points). Circulating clusterin in serum has been evaluated in a preliminary fashion using a Western blot technique, with results indicating decreased levels associated with OGX-

011 therapy. Further studies (e.g. antigen capture assay) will be performed to assess whether this will be a useful surrogate tissue/marker of biologic effect.

Based on these data, dose escalation for the trial was halted, and the recommended phase II dose was declared 640 mg based on the demonstration of biologic effect on clusterin expression. Furthermore, given that dose-limiting toxicities of oligonucleotides are potentially related to peak concentration and that complement activation in pre-clinical studies was predicted to occur at peak doses of ≥100 ug/ml, extrapolating from the current pharmacokinetic data did not justify further dose escalation.

Due to the delays in the start-up of the phase I clinical trial, a 1 year time extension is requested in order to complete the phase II (Task 3 and associated Task 4 elements) portion of this proposal.

KEY RESEARCH ACCOMPLISHMENTS

- First clinical trial of a second generation phosphorothioate antisense oligonucleotide in patients with cancer
- Novel study design using neoadjuvant therapy prior to radical prostatectomy
- Proof of principal demonstration of biologic effect
- Determination of recommended phase II dose of OGX-011 based on biological efficacy

REPORTABLE OUTCOMES

Manuscripts

1. Chi KN, Gleave ME. Antisense approaches in prostate cancer. Expert Opinion on Biologic Therapy. 4(6):927-936, 2004.

2. Chi KN, Eisenhauer E, Fazli L, Jones EC, Goldenberg SL, Gleave ME. A Phase I Pharmacokinetic and Pharmacodynamic Study of OGX-011, A 2'methoxyethyl Phosphorothioate Gapmer Antisense To Clusterin, In Patients With Localized Prostate Cancer Prior To Radical Prostatectomy. Manuscript in preparation for Journal of Clinical Oncology.

Abstracts

1. Chi KN, Eisenhauer E, Fazli L, Jones EC, Powers J, Ayers D, Goldenberg SL, Gleave ME. A Phase I Pharmacokinetic and Pharmacodynamic Study of OGS-011, A 2'methoxyethyl Phosphorothioate Gapmer Antisense To Clusterin, In Patients with Localized Prostate Cancer Prior To Radical Prostatectomy. Proc of ASCO, Abstract No. 3033, 2004. ***Poster Discussion Session***, New Orleans, June 2004.

2. Chi KN, Eisenhauer E, Fazli L, Jones EC, Powers J, Ayers D, Goldenberg SL, Gleave ME. A Phase I Pharmacokinetic and Pharmacodynamic Study of OGS-011, A 2'methoxyethyl Phosphorothioate Gapmer Antisense To Clusterin, In Patients with Localized Prostate Cancer Prior To Radical Prostatectomy. Canadian Urological Association, 59th Annual Meeting. ***Oral Presentation***, Podium Session II: Prostate Cancer, Whistler, June 2004.

3. Chi KN, Eisenhauer E, Fazli L, Jones EC, Powers J, Hurtado-Coll A, Goldenberg SL, Gleave ME.A phase I pharmacokinetic (PK) and pharmacodynamic (PD) study of OGX-011, a 2'methoxyethyl phosphorothioate antisense to *clusterin,* in patients with prostate cancer prior to radical prostatectomy. Accepted at the 16th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics. Accepted for ***Oral Presentation*** at the Proffered Papers Plenary Session, Geneva, September 2004.

Presentations

1. "Translating Molecular Mechanisms to Therapeutics". 2nd Asia-Pacific Society of Uro-oncology Meeting. Hong Kong, December 2003.

2. "Phase I Study of OGX-011 (Clusterin Antisense) and Neoadjuvant Hormone Therapy Prior to Radical Prostatectomy in Patients with Localized Prostate Cancer". Society of Urologic Oncology Program at the American Urological Association Annual Meeting, San Francisco, May 2004.

CONCLUSIONS

This phase I trial provides proof of principal evidence that OGX-011 can inhibit expression of clusterin in prostate cancer cells in humans. This is the first demonstration of dose dependent inhibition of a target, within target tissue by an antisense targeted therapeutic. Because of the successful determination of the biologically effective dose, phase II clinical trials with OGX-011 can move forward with confidence in the dosing regimen and schedule.

TABLES AND FIGURES

Table 1. Patient Characteristics

Characteristic		Number of Patients N=25
Median Age (Range)		63 (45-71)
Gleason Score	6	5
	7	14
	8-10	6
Baseline PSA	<10	16
	10-20	6
	>20	3
Clinical Stage	1c	12
	2a	9
	2b	3
	3a	1

Table 2. Hematologic Adverse Events

		GRADE			
DOSE LEVEL		*1*	*2*	*3*	*4*
40-80 mg	WBC	*1*			
(N=4)	ANC				
	Hgb	*2*			
	Platelets				
160 mg	WBC				
(N=3)	ANC				
	Hgb	*3*			
	Platelets				
320 mg	WBC	*4*			
(N=6)	ANC	*1*	*1*		
	Hgb	*4*			
	Platelets				
480 mg	WBC	*2*			
(N=6)	ANC	*1*			
	Hgb	*5*			
	Platelets	*1*			
640 mg	WBC	*2*			
(N=6)	ANC	*1*			
	Hgb	*5*			
	Platelets	*3*			

Table 3. Non-Hematologic Adverse Events

		GRADE			
		1	2	3	4
40-80 mg	AST	1	1		
(N=4)	ALT		1		
160 mg	Fatigue	2			
(N=3)	Rigors/Chills	1			
	Rhinitis	1			
	Headache	1			
	Creatinine	1			
320 mg	Fatigue	5			
(N=6)	Rigors/Chills	4			
	Fever	3			
	Nausea	2			
	Vomiting	1			
	Arthralgias	2			
	Myalgias	2			
	Dizziness	1			
	AST	3			
	ALT	3			
480 mg	Fatigue	2	1		
(N=6)	Rigors/Chills	6			
	Fevers	4	1		
	Arthralgias	1			
	Anorexia		1		
	Dyspepsia	1			
	Pruritis	1			
	Weight Gain	1			
	Creatinine	1			
	Alk Phos	1			
	AST	3	1		
	ALT	2			
640 mg	Fatigue	5			
(N=6)	Rigors/Chills	6			
	Fever	4	1		
	Myalgia	1			
	Headache	1			
	Diarrhea	2			
	Creatinine	1			
	Bilirubin	1			
	Alk Phos	1			
	AST	3	2		
	ALT	3	1		

Table 4. OGX-011 Plasma Pharmacokinetics

Dose Level	*OGX-011 Dose*	*Day*	*CMAX (µg/ml)*	*AUC (µg.h/ml)*	*T1/2 (hrs)*	*Cl (L/h)*	*MRT (hrs)*	*Vss (L)*
1	40 mg	1	4.00	9.32	0.61	4.27	2.52	10.88
		29	3.64	8.30	0.60	4.83	2.62	12.70
2	80 mg	1	12.31 ±4.60	33.96 ±12.00	0.96 ±0.12	2.59 ±1.05	2.63 ±0.23	6.71 ±2.12
		29	11.21 ±4.73	29.65 ±14.04	1.23 ±0.60	3.25 ±1.75	2.90 ±0.12	9.35 ±4.86
3	160 mg	1	23.15 ±2.70	70.98 ±13.82	2.03 ±1.58	2.04 ±0.64	3.03 ±0.57	7.93 ±2.92
		29	22.56 ±5.89	66.59 ±15.91	2.00 ±0.12	2.48 ±0.52	3.30 ±0.30	8.15 ±1.36
4	320 mg	1	40.23 ±10.39	133.22 ±17.30	1.98 ±0.42	2.32 ±0.37	3.13 ±0.23	8.21 ±0.60
		29	31.22 ±8.16	113.00 ±26.06	2.55 ±0.27	2.86 ±0.73	4.00 ±0.50	12.34 ±3.39
5	480 mg	1	56.31 ±15.71	195.84 ±46.95	2.09 ±0.29	2.39 ±0.51	3.13 ±0.16	8.83 ±1.78
		29	49.05 ±16.24	202.74 ±70.61	2.78 ±0.14	2.56 ±0.70	4.38 ±0.33	11.26 ±2.54
6	640 mg	1	61.11 ±5.52	236.99 ±31.51	2.20 ±0.33	2.58 ±0.26	3.20 ±0.24	10.23 ±1.76
		29	69.85 ±4.77	274.55 ±48.67	3.09 ±0.47	2.36 ±0.37	4.76 ±0.42	11.63 ±1.44

Figure 1. OGX-011 Maximum Concentrations (C_{max}) (Panel A) and Area Under The Curve (AUC) (Panel B) Vs. Dose Curves

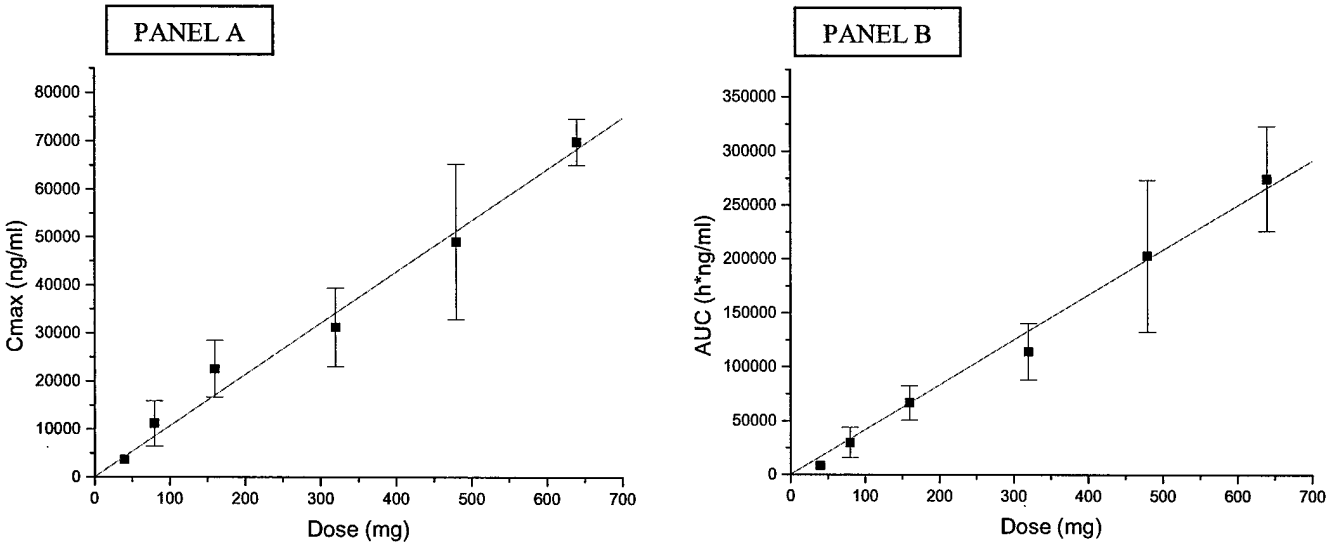


Figure 2. Scatter Plot of OGX-011 Tissue Concentrations Vs. Dose

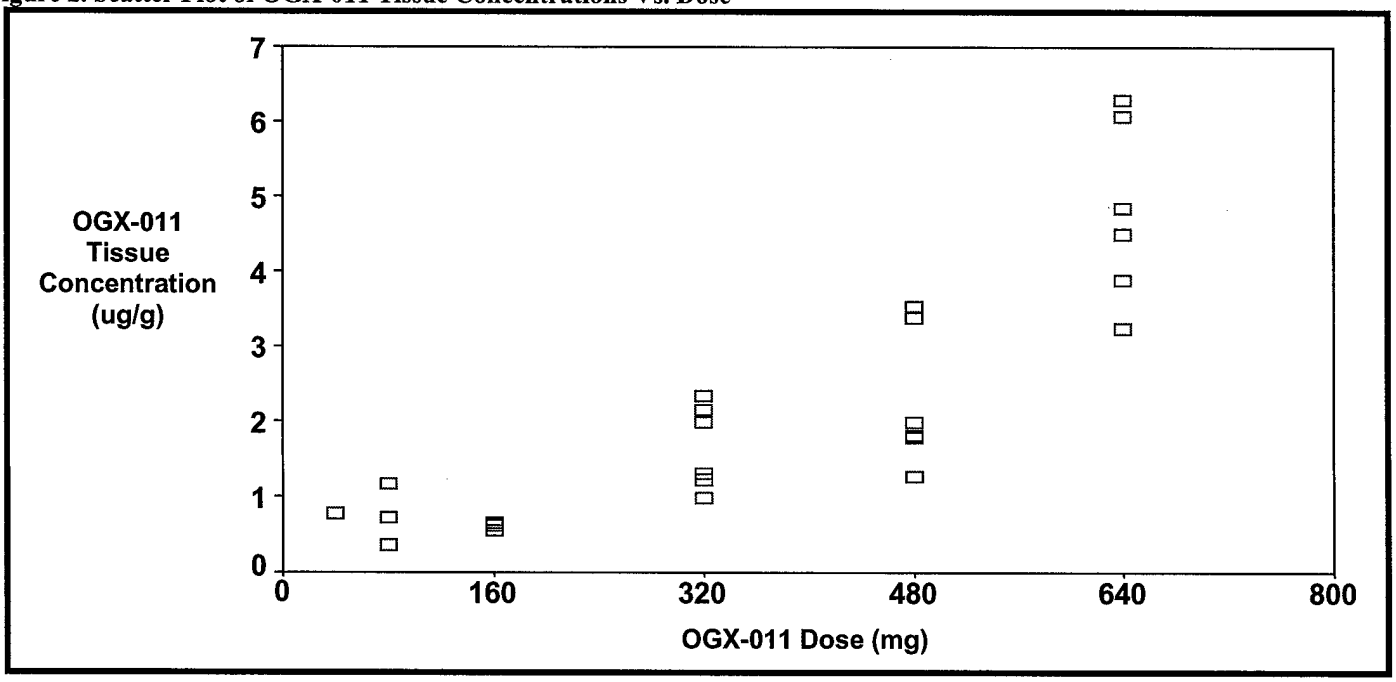


Figure 3. Clusterin mRNA Expression in Microdissected Prostate Cancer Cells (Quantitative Real Time-PCR)

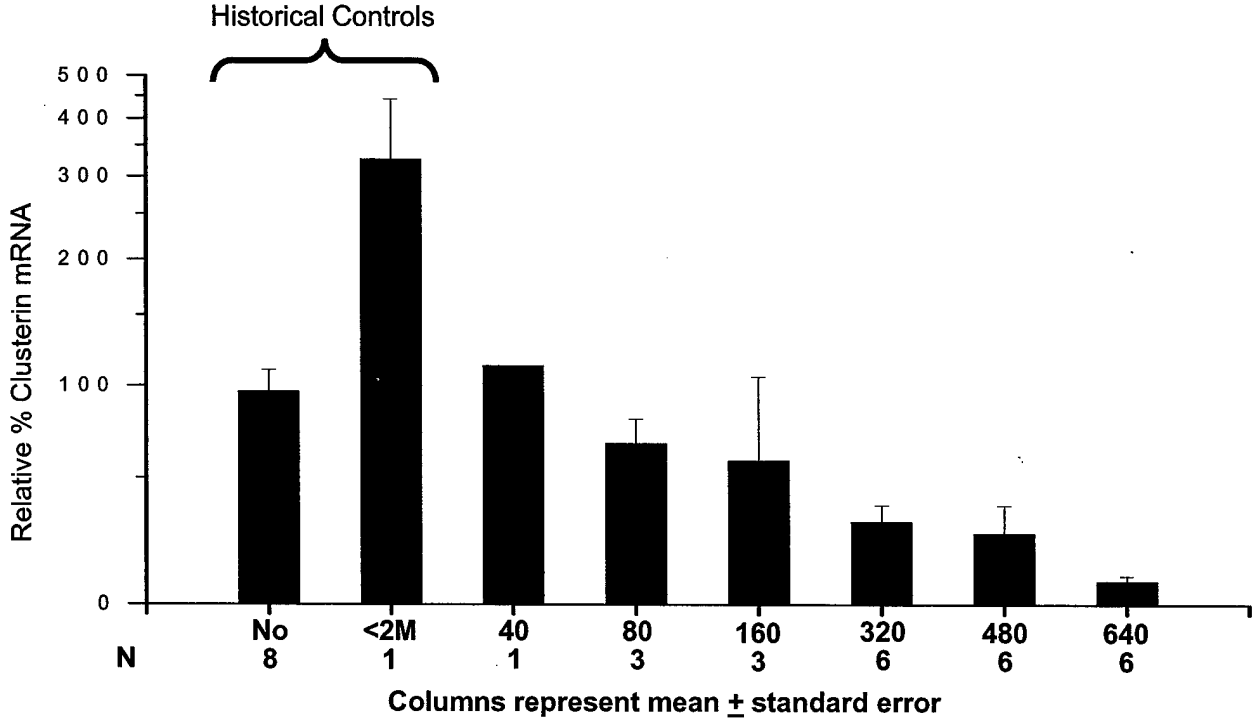


Figure 4. Clusterin Protein Expression in Prostate Cancer Cells (Immunohistochemistry - Overall Visual Score)

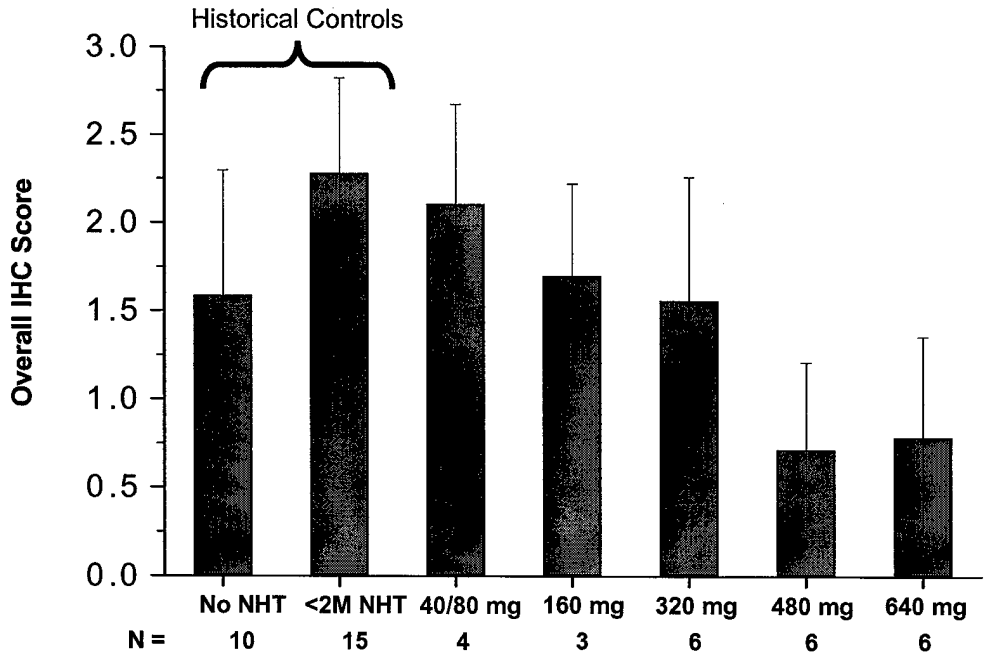


Figure 5. Box Plots of Clusterin Protein Expression in Prostate Cancer Cells (Immunohistochemistry - Percentage of Cells with a Visual Score 0)

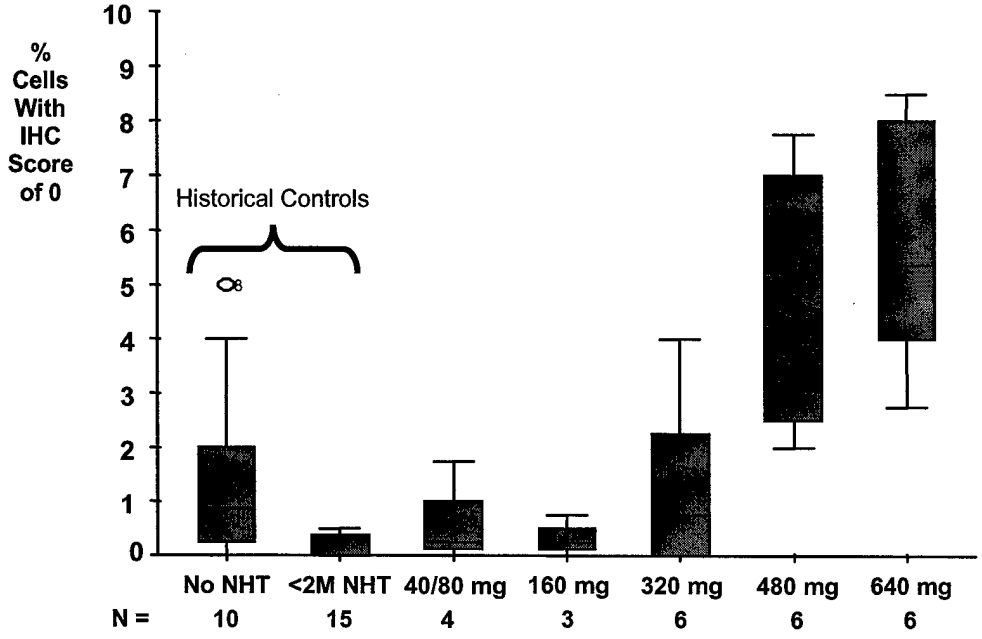


Figure 6. Change in Clusterin Protein Expression in Prostate Cancer Cells from Baseline to Prostatectomy (Immunohistochemistry - Percentage of Cells with a Visual Score 0)

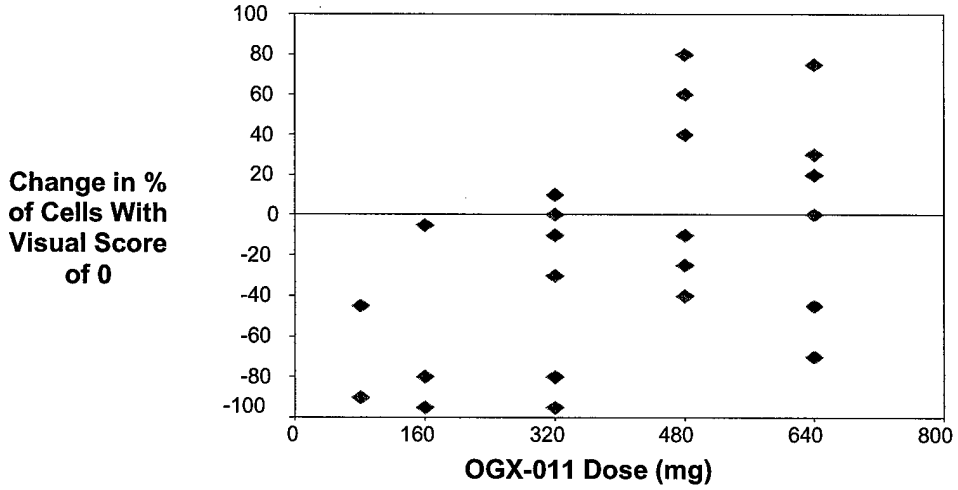


Figure 7. Clusterin mRNA Expression in Normal Lymph Nodes (Quantitative Real Time-PCR)

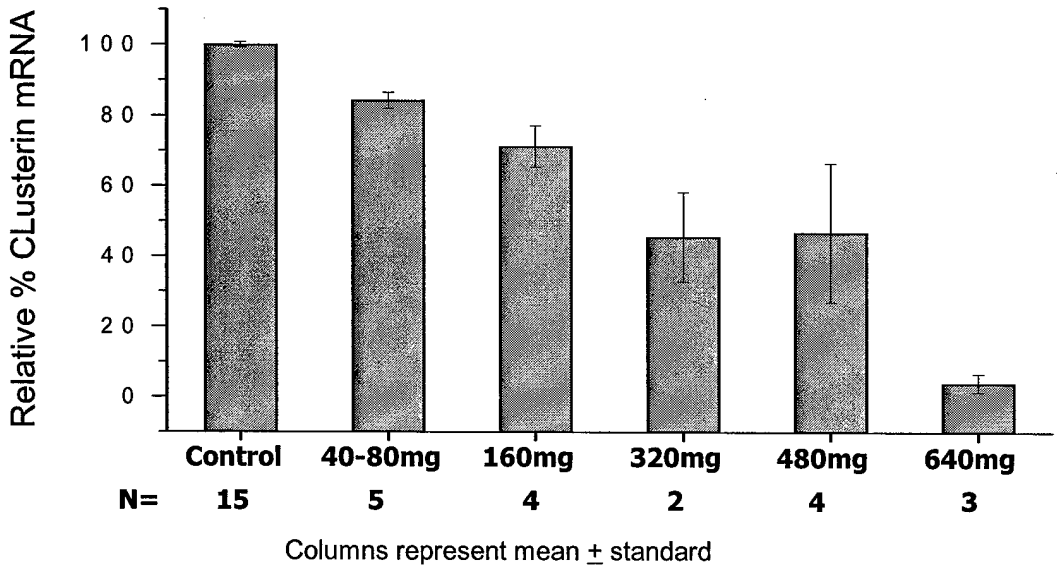
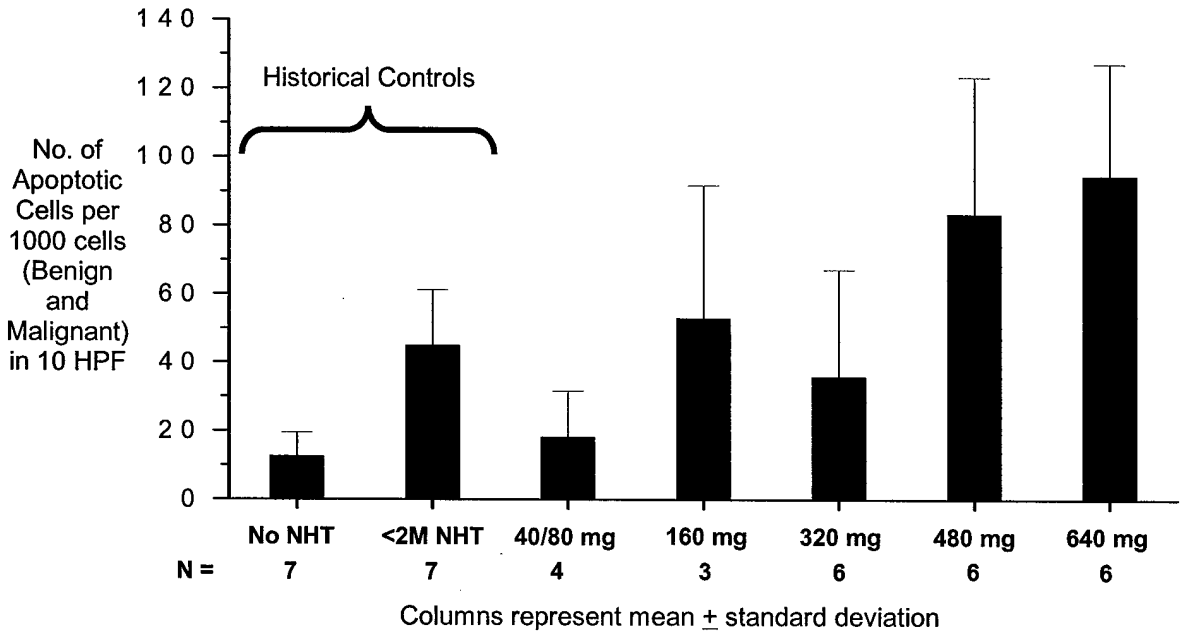


Figure 8. Apoptotic Index (Immunohistochemistry of ssDNA)



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APPENDIX

Meeting: 2004 ASCO Annual Meeting
Category: Developmental Therapeutics - Molecular Therapeutics
SubCategory: Gene Therapy/Antisense Strategies

Bookmark

A phase I pharmacokinetic (PK) and pharmacodynamic (PD) study of OGX-011, a 2'methoxyethyl phosphorothioate antisense to *clusterin,* in patients with prostate cancer prior to radical prostatectomy.

Abstract No: 3033

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Abstract:

Background: The *clusterin* gene encodes a chaperone protein that promotes cell survival. *Clusterin* is expressed in a variety of cancers including prostate, increases in response to after androgen abaltion and other apoptotic stimuli, and confers a resistant phenotype in pre-clinical models . OGX-011 (Oncogenex Technologies Inc) is a 2nd generation antisense complimentary to *clusterin* mRNA that inhibits expression of *clusterin* in xenograft models and thereby increases sensitivity to therapy. The objective of this first-in-man study was to determine phase II dose based on optimal target regulation effect in addition to standard toxicity parameters. **Methods:** Patients having localized prostate cancer with high-risk features and candidates for prostatectomy were enrolled to this dose escalation trial. OGX-011 was given by IV infusion over 2 hrs at a starting dose of 40mg on days 1, 3, 5, 8, 15, 22, and 29. Buserelin and flutamide were started on day 1. Prostatectomy was performed day 30-36. OGX-011 plasma PK and prostate tissue concentrations were determined. Prostate tissues, serial samples of peripheral blood mononuclear cells, and serum were assessed for *clusterin* expression. **Results:** 20 patients have been enrolled to 6 cohorts with doses of OGX-011 up to 640mg. Toxicity has been limited to grade 1 or 2, including fevers, rigors, fatigue and transient AST and ALT elevations. Plasma PK analysis showed linear increases in AUC with a t1/2 of approximately 2 hrs. OGX-011 prostate tissue concentrations increased with dose to a mean of 2.30 ug/g (~300nM) at 480mg dosing. Dose dependent decreases in prostate tumor *clusterin* expression were observed by immunohistochemistry (IHC) and in situ hybridization. By IHC, mean % cancer cells staining for 0 intensity at 480mg was 49.2% (SE 9.0) compared with 3.3-14.5% for lower dose levels. **Conclusions:** OGX-011 is well tolerated and can inhibit *clusterin* expression in prostate cancers. Dose escalation and additional PD assessments continue. Supported by a grant from the U.S. Department of Defense and coordinated by the NCIC-CTG.